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Contamination and Molecular Characterization of *Salmonella* spp. Isolated from Pig Carcasses and Pork Collected from Slaughterhouses and Retail Markets in Thai Binh Province

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Abstract

Pork is considered one of the major vehicles for *Salmonella* transmission to humans. This study aimed to determine the prevalence, serovar distribution, and virulence gene profiles of *Salmonella* isolates recovered from pig carcasses and pork in Thai Binh province. A total of 192 samples, comprising carcass swabs and pork samples, were collected from slaughterhouses and wet markets. *Salmonella* was detected in 22.92% (44/192) of the samples, with prevalence rates of 25.00% (24/96) in slaughterhouses and 20.83% (20/96) in wet markets. Among the 44 *Salmonella* isolates, only S. Typhimurium (31.82%, 14/44) and S. Virchow (4.55%, 2/44) were identified using the PCR-based serotyping assay targeting five selected serovars, whereas 63.63% (28/44) of the isolates remained untyped. The virulence genes *invA, msgA*, *tolC*, *lpfC*, *pagC*, and *spaN* were detected at relatively high frequencies of 100%, 90.91%, 77.27%, 72.73%, 70.45%, and 70.45%, respectively. In contrast, the *spvC* gene was found in only 4.5% of the isolates, whereas none carried the *cdtB* gene. The results of ESBL test showed that 27.27% (12/44) of the isolates were ESBL producers carrying $bla_{CTX-M-1}$, $bla_{CTX-M-9}$, and bla_{TEM} genes. The detection of ESBL-producing *Salmonella* in carcasses and pork highlights a potential public health risk and underscores the need for enhanced food safety surveillance and intervention measures to reduce *Salmonella* contamination.

Keywords

Salmonella, virulence gene, ESBLs, pork, carcass, antibiotic resistance

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Introduction

Salmonella is one of the leading foodborne pathogens responsible for severe illnesses in humans (Zhao *et al.*, 2001). Non-typhoidal

Salmonella is estimated to cause approximately 93.8 million human infections and 155,000 deaths annually worldwide, of which nearly 85% are associated with the consumption of contaminated food (Chlebicz & Śliżewska, 2018). Clinical manifestations of salmonellosis include gastroenteritis, abdominal cramps, bloody diarrhea, fever, myalgia, headache, and nausea (Ehuwa *et al*., 2021). Immunocompromised individuals, infants, and older adults are particularly susceptible to severe complications, including septicemia and invasive infections such as meningitis, osteomyelitis, and septic arthritis (Lamichhane *et al*., 2024). Owing to its widespread occurrence and substantial public health impact, *Salmonella* has become a priority pathogen in surveillance programs implemented by many countries to reduce its incidence and interrupt transmission from food-producing animals to humans through contaminated food products (EFSA, 2026; Mkangara, 2023).

Pork is recognized as one of the major vehicles for *Salmonella* transmission to humans and has been implicated in numerous foodborne outbreaks worldwide (Algino *et al*., 2009; De Knegt *et al*., 2015). Contamination of pork may occur through fecal contamination of infected animals during slaughter and processing (Soliani *et al*., 2023). The pathogenicity of *Salmonella* depends largely on the serovar and the repertoire of virulence genes that it carries. These genes, located on *Salmonella* pathogenicity islands and virulence plasmids, play essential roles in bacterial adhesion, invasion, intracellular survival, and systemic dissemination. For example, genes encoded on *Salmonella* pathogenicity island 1 (SPI-1) mediate epithelial cell invasion, whereas plasmid-borne virulence genes contribute to systemic infection (Foley & Lynne, 2008). In addition to virulence, antimicrobial resistance in *Salmonella* has emerged as a major global public health concern, particularly the increasing occurrence of extended-spectrum β-lactamase (ESBL)-producing strains. These strains exhibit resistance to third- and fourth-generation cephalosporin, which are classified by the World Health Organization as critically important or highly important antimicrobials for the treatment of salmonellosis (WHO, 2017). Consequently, infections caused by ESBL-producing *Salmonella* present considerable therapeutic challenges (Campos *et al*., 2019). Continuous monitoring of the occurrence, virulence characteristics, and antimicrobial resistance profiles of *Salmonella* throughout the pork production chain is therefore essential for food safety risk assessment and the development of effective control strategies.

Thai Binh Province is one of the major pig-producing regions in northern Vietnam, where pork production and distribution rely predominantly on small-scale slaughterhouses and traditional wet markets. Previous studies conducted in Vietnam have demonstrated that inadequate hygiene practices during slaughtering, transportation, and retail display, together with poor environmental sanitation and the absence of an effective cold chain, facilitate contamination of pork with foodborne pathogens, particularly *Salmonella* (Dang-Xuan *et al*., 2019; Ngo *et al*., 2021). Within traditional pork value chains, cross-contamination originating from slaughter facilities, equipment, workers, and retail environments has been identified as a major route for the dissemination of *Salmonella* (Podolak *et al*., 2010). However, information regarding the prevalence, serovar distribution, virulence characteristics, and ESBL production of *Salmonella* isolated from pork and pig carcasses in Thai Binh Province remains limited. Therefore, this study aimed to investigate the prevalence, serovar distribution, virulence gene profiles, and ESBL production of *Salmonella* isolates recovered from pork and pig carcasses collected from slaughterhouses and retail markets in Thai Binh Province.

Materials and Methods

Materials

The chemicals and culture media used in this study included Buffered Peptone Water (Oxoid, UK), Rappaport–Vassiliadis Soya Peptone Broth (Merck, Germany), Müller–Kauffmann Tetrathionate Novobiocin Broth (Merck, Germany), Bismuth Sulfite Agar (HiMedia,

India), Xylose Lysine Deoxycholate Agar (HiMedia, India), Nutrient Broth (Merck, Germany), the API 20E identification kit (bioMérieux Vitek Inc., Hazelwood, MO, USA), glycerol (Merck, Germany), the DNeasy Blood & Tissue Kit (Qiagen, Germany), DreamTaq Green PCR Master Mix (2×) (Thermo Fisher Scientific, USA), sequence-specific primers (Phu Sa Genomics, Vietnam), DNA ladder (Thermo Scientific, USA), ethidium bromide, sodium EDTA, Tris hydrochloride (Bio Basic, Canada), UltraPure™ water, agarose (Sigma-Aldrich, USA), and 10× TBE buffer (Bio Basic, Canada). The antimicrobial disks used for ESBL detection were ceftazidime (30µg, Oxoid, UK), ceftazidime/clavulanic acid (30/10µg, Oxoid, UK), cefotaxime (30 µg, Oxoid, UK), and cefotaxime/clavulanic acid (30/10µg, Oxoid, UK).

Methods

Sample collection

A total of 96 pork samples and 96 carcass swab samples were collected from 24 traditional wet markets and 69 slaughterhouses in Thai Binh Province between January and August 2025, yielding a total of 192 samples. At each slaughterhouse, one to three carcass swab samples were collected, whereas four pork samples were obtained from each wet market.

Sampling for microbiological analysis was performed in accordance with the Vietnamese technical regulation QCVN 01-04:2009. For carcass sampling, sterile cotton swabs were pre-moistened with 10mL of phosphate-buffered saline (PBS) before use. A sterile stainless-steel template (10 × 10cm) was placed on four anatomical sites of each carcass (ham, back, chest, and cheek) to define the sampling area. Using sterile forceps, the swab was rubbed vertically, horizontally, and diagonally across each designated area with 10 strokes in each direction over a period of at least 20s. After sampling, the swab was transferred to a sterile stomacher bag containing 25mL of PBS.

For pork sampling, approximately 20g of tissue was aseptically collected from each of five pork cuts (shoulder, loin, belly, ham, and leg) and pooled to obtain a composite sample of approximately 100g. Each sample was placed in a sterile sampling bag, appropriately labelled, and transported to the laboratory in insulated containers maintained at approximately 4°C. All samples were processed for *Salmonella* isolation within 24h of collection.

Isolation of Salmonella

Isolation of *Salmonella* was conducted following TCVN 10780-1:2017 (ISO 6579-1:2017). For pork samples, 25g of meat was aseptically cut into small pieces and homogenized with 225mL of Buffered Peptone Water (BPW). For carcass swab samples, the entire swab suspension in PBS was transferred into 225mL of BPW. The pre-enrichment cultures were incubated at 37°C for 18h. During the selective enrichment stage, the sample was transferred into Rappaport–Vassiliadis Soy Broth (RVS) and Muller–Kauffmann tetrathionate novobiocin broth (MKTTn). The RVS broth was then kept at 41.5°C for 24h, while the MKTTn broth was stored at 37°C for 24h. Following enrichment, cultures were streaked onto two selective agar media, Xylose Lysine Deoxycholate agar (XLD) and Bismuth Sulphite agar (BS), and stored at 37°C for 24h. Presumptive *Salmonella* colonies from XLD and BS plates were subsequently subcultured onto Tryptic Soy Agar (TSA). The plates were then incubated at 37°C for 24h. Presumptive isolates were then confirmed using the API 20E kit (bioMérieux Vitek, Inc., Hazelwood, MO, USA). Finally, confirmed *Salmonella* isolates were preserved in Microbank™ tubes (Pro-Lab Diagnostics, Canada) at -80°C for further analysis.

Serovar identification

The DNeasy Blood & Tissue Kit (Qiagen) was used to extract DNA of the isolates according to the manufacturer's instructions. Serovar identification of the *Salmonella* isolates was carried out using the PCR technique as described by Chiang *et al.* (2018). The thermal cycling conditions consisted of an initial denaturation at 94°C for 2min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 59°C for 30s, and extension at 72°C for 30s, with a final extension at 72°C for 7min.

Table 1. Information on primer pairs used for serovar identification of *Salmonella* isolates.

No.	Serovars	Target genes	Primer	Sequence (5'-3')	Size (bp)	Reference
1	Enteritidis	*Prot6e*	ENTE-F	ATATCGTCGTTGCTGCTTCC	206	Chiang *et al.* (2018)
			ENTE-R	CATTGTTCCACCGTCACTTTG		
2	Typhimurium	*MDH*	TYPH-F	CGCATTCCACCACGCCCTTC	261	
			TYPH-R	TGCCAACGGAAGTTGAAGTG		
3	Hadar	*HSR3*	HADA-F	CCTCAAATTAATGCCAGAGAG	427	
			HADA-R	GTAGCAATTTATGGCTTACTACA		
4	Infantis	*ISR2-ISR3*	NFA2-F	TGAGGGCGAGAGGTATTGTTAT	240	
			In-Vi Uni-R	ATACGATACTACAATACCCGACG		
5	Virchow	*Hypothetical protein*	VIRC1-F	ATTGTTTATGCATAGGCCGAC	273	
			In-Vi Uni-R	ATACGATACTACAATACCCGACG		

Detection of virulence genes in Salmonella isolates

Virulence genes of *Salmonella* isolates were detected using multiplex PCR following the method previously described by Chiu & Ou (1996) and Bahramianfard *et al*. (2021). Information on the primer pairs used for virulence gene detection is presented in **Table 2**. The amplification conditions of the *invA* gene consisted of an initial denaturation at 94°C for 60s, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 56°C for 30s, and extension at 72°C for 2min, with a final extension at 72°C for 10min. For the amplification of *pagC, cdtB, msgA, spaN, tolC,* and *lpfC* genes, the thermal conditions included an initial denaturation at 95°C for 2min, followed by 35 cycles of denaturation at 94°C for 40s, annealing at 62°C for 30s, and extension at 72°C for 40 s, with a final extension at 72°C for 10 min. For detection of the *spvC* gene, the PCR conditions consisted of an initial denaturation at 94°C for 2min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 57°C for 40s, and extension at 72°C for 30s, with a final extension at 72°C for 10min.

Detection of phenotypic and genotypic characteristics of ESBL-producing Salmonella isolates

Phenotypic screening for ESBL production was performed by the ESBL test according to the CLSI guideline, with cefotaxime and ceftazidime disks alone and in combination with clavulanic acid (CLSI, 2024). Briefly, bacterial suspension (0.1mL; 10^8 CFU mL^{-1}) was evenly spread onto Mueller–Hinton Agar (MHA) plates. Antibiotic disk pairs, including ceftazidime (30µg, Oxoid) and ceftazidime/clavulanic acid (30/10µg, Oxoid), as well as cefotaxime (30µg, Oxoid) and cefotaxime/clavulanic acid (30/10µg, Oxoid), were then positioned on the agar surface, followed by incubation at 37°C for 16-18h. Isolates were identified as ESBL producers when the inhibition zone diameter around the clavulanic acid-containing disk was at least 5 mm greater than that without clavulanic acid.

ESBL-encoding genes in the *Salmonella* isolates were subsequently detected by multiplex PCR as previously described (Le *et al*., 2015). The primer pairs used in this study are presented in **Table 3**. The PCR program included an initial denaturation step at 95°C for 5min, followed by 25 cycles of denaturation at 95°C for 30s, annealing at 60°C for 90s, and extension at 72°C for 90s, with a final extension step at 68°C for 10min.

Statistical analysis

Data were analyzed using Microsoft Excel (2021, Microsoft, USA). Differences in *Salmonella* prevalence rates between pork and carcass were evaluated using Pearson's chi-square test with IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Table 2. Primer pairs used for virulence gene detection in *Salmonella* isolates

No.	Target genes	Primer	Primer sequences (5’-3’)	Size (bp)	Reference
1	*invA*	invA-1	ACAGTGCTCGTTTACGACCTGAAT	244	Chiu & Ou (1996)
		invA-2	AGACGACTGGTACTGATCGATAAT		
2	*pagC*	pagC-F	CGCCTTTTCCGTGGGGTATGC	454	Bahramianfard *et al.* (2021)
		pagC-R	GAAGCCGTTTATTTTTGTAGAGGAGATGTT		
3	*cdtB*	cdtB-F	ACAACTGTCGCATCTCGCCCCGTCATT	268	
		cdtB-R	CAATTTGCGTGGGTTCTGTAGGTGCGAGT		
4	*msgA*	msgA-F	GCCAGGCGCACGCGAAATCATCC	189	
		msgA-R	GCGACCAGCCACATATCAGCCTCTTCAAAC		
5	*spaN*	spaN-F	AAAAGCCGTGGAATCCGTTAGTGAAGT	504	
		spaN-R	CAGCGCTGGGGATTACCGTTTTG		
6	*tolC*	tolC-R	TACCCAGGCGCAAAAAGAGGCTATC	161	
		tolC-F	CCGCGTTATCCAGGTTGTTGC		
7	*lpfC*	lpfC-F	GCCCCGCCTGAAGCCTGTGTTGC	641	
		lpfC-R	AGGTCGCCGCTGTTTGAGGTTGGATA		
8	*spvC*	spvC-F	ACTCCTTGCACAACCAAATGCGGA	571	
		spvC-R	TCTCTTCTGCATTTCGCCACCATCA		

Table 3. Information on the primer pairs used for the detection of ESBL-encoding genes

No.	Target genes	Primer	Primer sequence (5'-3')	Size (bp)	Reference
1	bla_{TEM}	TEM-410F	GGTCGCCGCATACACTATTCTC	372	Le *et al.* (2015)
		TEM-781R	TTTATCCGCCTCCATCCAGTC		
2	bla_{SHV}	SHV-287F	CCAGCAGGATCTGGTGGACTAC	231	
		SHV-517R	CCGGGAAGCGCCTCAT		
3	$bla_{CTX-M-1}$	CTXM-1-115F	GAATTAGAGCGGCAGTCGGG	588	
		CTXM-1-702R	CACAACCCAGGAAGCAGGC		
4	$bla_{CTX-M-2}$	CTXM-2-39F	GATGGCGACGCTACCCC	107	
		CTXM-2-145R	CAAGCCGACCTCCCGAAC		
5	$bla_{CTX-M-9}$	CTXM-9-16F	GTGCAACGGATGATGTTCGC	475	
		CTXM-9-490R	GAAACGTCTCATCGCCGATC		
6	$bla_{CTX-M-8/25}$	CTXM-8g25g-533F	GCGACCCGCGCGATAC	186	
		CTXM-8g25g-718R	TGCCGGTTTTATCCCCG		

Results and Discussion

Prevalence rate of *Salmonella* in carcass and pork samples

The results presented in **Table 4** show that *Salmonella* was detected in 22.9% (44/192) of the samples collected in Thai Binh Province. The prevalence of *Salmonella* was 25.0% (24/96) in carcass swab samples collected from slaughterhouses and 20.8% (20/96) in pork samples collected from retail markets.

In the present study, no significant difference was observed in the prevalence of *Salmonella* between carcass swab and pork samples (P >0.05). This finding is consistent

Table 4. Prevalence rate of *Salmonella* in pig carcass and pork samples

No.	Sampling site	Sample type	No. of samples	No. of positive samples	Prevalence (%)
1	Slaughterhouses	Carcass swab	96	24	25
2	Markets	Pork	96	20	20.83
	Total		192	44	22.92

with that reported by Holohan *et al*. (2022), who found similar *Salmonella* prevalence rates in carcasses (33.4%) and pork (35.4%) collected from Hung Yen and Nghe An provinces. In contrast, Tran Thi Nhat *et al*. (2019) reported a significantly higher prevalence of *Salmonella* in pig carcasses (73.3%) than in pork (55.0%) in Nghe An Province. Conversely, Le Hong Phong *et al*. (2019) reported a lower prevalence in carcasses (14.4%) than in pork (27.8%) in the Mekong Delta region. These discrepancies suggest that the prevalence of *Salmonella* may vary considerably according to slaughter hygiene, transportation conditions, retail practices, and regional production systems.

The relatively high prevalence of *Salmonella* in carcass samples observed in this study may be attributed to hygienic deficiencies during slaughter. Procedures such as scalding, dehairing, evisceration, carcass splitting, and cutting can facilitate fecal contamination and cross-contamination between carcasses when hygienic practices are inadequate. In addition, contaminated equipment, processing surfaces, and workers' hands may further contribute to the dissemination of *Salmonella* within slaughterhouses. Infected pigs also represent the primary reservoir of *Salmonella*, providing a continuous source of contamination during processing. Previous studies have demonstrated that most carcass contamination occurs during slaughter, and cross-contamination may approach 100% under poor hygienic conditions (Arguello *et al*., 2013; Botteldoorn *et al*., 2003). These factors may explain the relatively high prevalence of *Salmonella* detected in carcass swab samples in the present study.

Despite the multiple opportunities for contamination during slaughter, the similar prevalence observed between carcass swabs and pork samples may reflect changes occurring during post-slaughter handling and retail distribution (Giovannini *et al*., 2004). In Thai Binh Province, carcasses are commonly transported to traditional markets without refrigeration and displayed at ambient temperature. Under these conditions, the rapid proliferation of competing background microflora on meat surfaces may suppress the growth of *Salmonella*. Moreover, environmental stresses, including temperature fluctuations, light exposure, and reduced moisture, may decrease bacterial viability or induce sublethal injury, thereby reducing the recovery of *Salmonella* during culture-based isolation (Ehuwa *et al*., 2021).

Overall, the prevalence of *Salmonella* observed in this study (22.9%) was lower than that reported in several previous studies conducted in Vietnam, where prevalence rates ranging from 44% to 64% have been reported in pork samples collected from Hanoi, Bac Ninh, Nghe An, and Ho Chi Minh City (Tran Thi Nhat *et al*., 2019; Truong *et al*., 2021). This lower prevalence may be attributed to differences in geographical location, sampling strategy, study period, sample type, and improvements in hygiene and food safety practices during slaughter and meat handling. Nevertheless, the prevalence observed in the present study was comparable with those reported in Mexico (17.3%) and Romania (22.6%) (Miranda *et al*., 2009; Tîrziu *et al*., 2020). By comparison, substantially lower prevalence rates have been reported in developed countries. For example, the European Food Safety Authority reported that only 1.85-2.15% of pig carcasses tested in Europe were positive for *Salmonella* (EFSA, 2018).

In general, the findings of the present study indicate that pork produced and marketed in Thai Binh Province remains a potential source of *Salmonella* infection for consumers. The detection of *Salmonella* in samples collected from both slaughterhouses and retail markets suggests that contamination may persist

throughout the pork production and distribution chain. These findings highlight the importance of strengthening hygiene management and implementing effective control measures at critical points throughout the production chain to reduce *Salmonella* contamination and improve food safety.

Serovar identification

The results of serovar identification are presented in **Table 5**. Of the five *Salmonella* serovars targeted by the multiplex PCR assay, only *S.* Typhimurium and *S.* Virchow were detected. Specifically, *S.* Typhimurium was identified in 14 isolates (31.8%), whereas *S.* Virchow was detected in only 2 isolates (4.5%). The remaining 28 isolates (63.6%) could not be assigned to any of the five targeted serovars and were therefore classified as not determined (ND). Although this method has high specificity, it should only be applied when the prevalence of strains in the study area is well known, and it is not suitable for identifying many serovars at once, as the number of *Salmonella* serovars can exceed 2500.

The predominance of *S.* Typhimurium observed in this study is consistent with previous reports from Vietnam and other countries, where this serovar is commonly detected in pigs and pork products (Bai *et al*., 2015; Broadway *et al*., 2021; Lai Thi Lan Huong & Trinh Dinh Thau, 2017; Tran Thi Nhat *et al*., 2019). The detection rate of *S.* Typhimurium in this study is of particular concern from a food safety perspective, as this serovar is recognized globally as one of the most important serovars for humans. Besides, *S.* Virchow, a serovar that has only infrequently been reported in pork and pork-derived products, has been detected in this study. Despite its relatively low incidence, *S.* Virchow remains of considerable public health concern owing to its documented association with human Salmonellosis outbreaks and its propensity to exhibit multidrug-resistant phenotypes (Bonalli *et al*., 2011; Uzairue *et al*., 2023). Collectively, these findings indicatee the substantial diversity and geographic variability of *Salmonella* serovars associated with pork and pork-derived products worldwide.

Detection of virulence genes in *Salmonella* isolates

Table 6 reveals the virulence genes in *Salmonella* isolates. All 44 (100%) isolates carried the *invA*. In addition, the genes *msgA*, *tolC*, *lpfC*, *pagC*, and *spaN* were commonly detected, with prevalence rates of 90.91%, 77.27%, 72.73%, 70.45%, and 70.45%, respectively. On the other hand, the *spvC* gene was found in only 4.55% of the isolates, while the *cdtB* gene was not present in any of the isolates.

The virulence gene profile observed in this study was generally consistent with previous reports. Nguyen Thi Hoai Thu *et al*. (2018) found that *S.* Enteritidis and *S.* Typhimurium isolates from retail meat in Hanoi carried virulence genes such as *invA* and *pagC*. Similarly, Punchihewage *et al*. (2023) reported that *Salmonella* isolates frequently harbored the *invA* (95%) and *pagC* (89%) genes, whereas the *spvC* gene was detected at a relatively lower frequency (6.6%). Notably, 4.2% of the isolates concurrently harbored all three virulence genes. Lozano-Villegas *et al*. (2023) observed relatively high detection rates of *msgA*, *pagC*, *tolC*, *lpfC*, and

Table 5. Serovar identification of *Salmonella* isolates (n = 44)

No.	Serovar	No. of positive samples	Prevalence (%)
1	Enteritidis	0	0
2	Typhimurium	14	31.82
3	Hadar	0	0
4	Infantis	0	0
5	Virchow	2	4.55
6	Not determined	28	63.63
	Total	44	100

Table 6. Virulence-associated gene profile in *Salmonella* isolates (n = 44)

No.	Virulence gene	No. of positive samples	Prevalence (%)
1	*invA*	44	100
2	*pagC*	31	70.45
3	*msgA*	40	90.91
4	*spaN*	31	70.45
5	*tolC*	34	77.27
6	*lpfC*	32	72.73
7	*spvC*	2	4.55
8	*cdtB*	0	0

spaN in *Salmonella* isolates from poultry, at 97.4%, 97.4%, 92.3%, 87.2%, and 79.5%, respectively. The variation in the prevalence of virulence genes across studies may be attributed to differences in sample sources, environmental conditions, and the genetic diversity of *Salmonella* strains across regions.

Phenotyping and genotyping of ESBL-producing *Salmonella* isolates

A total of 12 (27.27%) out of 44 *Salmonella* isolates were found to produce ESBLs. Of these, 4 isolates belonged to serovar Typhimurium, one isolate was identified as Virchow, and 7 isolates were not assigned to any of the five targeted serovars (**Table 7**). The prevalence of ESBL-producing *Salmonella* in food has been reported worldwide. In the present study, the incidence of ESBL-producing *Salmonella* (27.27%) was lower than that reported by Nadimpalli *et al.* (2019) in Cambodia. This finding may reflect the increasing antimicrobial selective pressure associated with antibiotic use in livestock production systems. The predominance of *S.* Typhimurium among ESBL-producing isolates is consistent with previous studies identifying this serovar as a major reservoir of antimicrobial resistance genes, particularly ESBL determinants, in both animal production and food systems (Campos *et al*., 2019). *S.* Typhimurium is well recognized for its broad host range, environmental adaptability, and capacity to acquire mobile resistance elements. In contrast, the detection of ESBL-producing *S.* Virchow is also of concern, as this serovar has increasingly been associated with invasive human infections and resistance to critically important antimicrobials (Uzairue *et al*., 2023).

The ESBL-encoding gene profile of the isolates is presented in Table 7. Specifically, two isolates harbored only the $bla_{CTX\text{-}M\text{-}1}$, whereas six were positive for $bla_{CTX\text{-}M\text{-}9}$. Notably, four isolates simultaneously carried both $bla_{CTX\text{-}M\text{-}1}$ and bla_{TEM}. Interestingly, ESBL-producing *Salmonella* isolates also carried multiple virulence-associated genes. Among them, isolates SALM9 (*S.* Typhimurium), SALM11 (*S.* Virchow), and SALM16 (not assigned to the five targeted serovars) exhibited the broadest virulence profiles (**Table 7**). Another notable finding was the high prevalence of the *tolC* gene among ESBL-positive isolates (10/12 isolates). The *tolC* gene encodes an outer membrane protein involved in multidrug efflux systems and may contribute to enhanced bacterial survival under antimicrobial stress conditions (Horiyama *et al*., 2010). The predominance of $bla_{CTX\text{-}M}$ observed in this study is in accordance with previous report describing CTX-M-type enzymes as the dominant ESBL genotype among *Enterobacteriaceae*, replacing the previously prevalent TEM and SHV variants (Bevan *et al*., 2017). The coexistence of ESBL production and virulence-associated determinants may reduce treatment efficacy and facilitate the persistence of *Salmonella* in food matrices.

To the best of our knowledge, information regarding ESBL-producing *Salmonella* in pork and carcass samples from Thai Binh is limited. Therefore, the present findings provide valuable baseline data for monitoring antimicrobial resistance and highlight the potential public

Table 7. Distribution of virulence and ESBL-encoding genes of ESBL-producing *Salmonella* isolates

No.	Isolate ID	Samples	Serovar	Virulence gene								ESBL genes
				invA	*pagC*	*cdtB*	*msgA*	*spaN*	*tolC*	*lpfC*	*spvC*	
1	SLC2	Pork	Typhimurium	+	-	-	+	+	+	+	+	$bla_{CTX\text{-}M\text{-}1}$
2	SLC3		ND	+	-	-	-	+	+	+	-	$bla_{CTX\text{-}M\text{-}9}$
3	SLC6		ND	+	-	-	+	+	+	+	-	$bla_{CTX\text{-}M\text{-}9}$
4	SLC8		ND	+	-	-	+	-	+	+	-	bla_{TEM}, $bla_{CTX\text{-}M\text{-}1}$
5	SLC12		ND	+	+	-	+	-	-	-	-	bla_{TEM}, $bla_{CTX\text{-}M\text{-}1}$
6	SLC16		Typhimurium	+	-	-	+	+	+	+	-	$bla_{CTX\text{-}M\text{-}9}$
7	SLC17		ND	+	+	-	+	+	+	+	-	$bla_{CTX\text{-}M\text{-}9}$
8	SALM2	Carcass swab	ND	+	+	-	+	-	-	+	-	bla_{TEM}, $bla_{CTX\text{-}M\text{-}1}$
9	SALM9		Typhimurium	+	+	-	+	+	+	+	-	$bla_{CTX\text{-}M\text{-}9}$
10	SALM11		Virchow	+	+	-	+	+	+	+	-	$bla_{CTX\text{-}M\text{-}9}$
11	SALM16		ND	+	+	-	+	+	+	+	-	$bla_{CTX\text{-}M\text{-}1}$
12	SALM21		Typhimurium	+	+	-	+	+	+	-	-	bla_{TEM}, $bla_{CTX\text{-}M\text{-}1}$

Note: ND: Not determined.

health risks associated with the dissemination of ESBL-producing *Salmonella* through the food chain. These findings also underscore the need for continuous surveillance and improved food safety measures.

Conclusions

Salmonella was detected at relatively high rates in pig carcass and pork samples collected in Thai Binh province. *S.* Typhimurium and *S.* Virchow were identified by PCR, while most isolates were not assigned to any of the five targeted serovars. All isolates carried at least one virulence-associated gene. Among the *Salmonella* isolates, a high proportion were identified as ESBL producers harboring ESBL-encoding genes. These findings indicate that pig carcasses and pork in Thai Binh may serve as reservoirs of potentially pathogenic and antimicrobial-resistant *Salmonella*, posing a public health concern. Improved hygiene and control measures throughout slaughtering, processing, and distribution are needed to reduce contamination and enhance food safety.

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